REMARKS

Applicant thanks the Examiner for her consideration of applicant's April 18, 2008 Response and her withdrawing of all of the previous rejections.

The Claim Amendments

Claims 45-50 and 57 are pending.

Applicant has amended claims 45 and 46 to clarify that the probes are derived from genomic DNA that is specific to at least one of the nucleic acid marker sequences. As explained in the specification (e.g., at page 27, lines 18-28), the marker sequence is used to identify a complete gene sequence, and probes are designed from that gene sequence.

Applicant has also amended claims 47-49 to define the recited levels of correlation. The amendment is supported in the specification, e.g., at page 29, lines 15-17.

Applicant has also amended claim 57 to correct an informality.

Office Action

Claim Objections

Claim 57 stands objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim should refer to other claims in the alternative. Applicant has amended claim 57 to correct the informality.

Rejections

35 U.S.C. §112, first paragraph – Enablement

Claims 45-50 and 57 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the enablement requirement. The Examiner contends that the claims contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner argues that the specification has no working examples of how to obtain a correlation between an abundance of a nucleic acid marker sequence and an abundance of an environmental parameter, and further contends that the guidance provided by the specification amounts to a mere invitation for the skilled artisan to try and follow the disclosed instructions to make and use the claimed invention. Further, the Examiner contends that the art to which the invention is directed is unpredictable and that it is not possible to reliably determine the abundance of different organisms in environmental samples and, therefore, to correlate such abundances to environmental parameters. Applicant respectfully traverses.

The application enables the skilled worker to practice the full scope of the pending claims. Example 3 (specification, page 44-46) provides a specific working example demonstrating that distinct nucleic acid tags, produced by the methods of this invention, distinguish samples taken from diverse locations in an environmental region, here the Rocky Mountain Oil Field Testing Center. The relative abundance of distinct, non-identical tags in each of the samples (see Figure 17) was determined. Further, 26 (or 45%) of the non-identical tags in the first sample (Wy-1) were not present in the second sample (Wy-2), and conversely, 47 (or 59%) of the non-identical tags in the second sample (Wy-2) were not present in the first sample (Wy-1). This result belies the Examiner's concern that it is not possible to characterize different samples using the tags of this invention.

Example 3 concludes by disclosing to the skilled worker that the 73 different tags (26 + 47) that characterize the samples are candidates for identifying DNA sequences whose presence and abundance correlates with the various parameters associated with each sample. Because Example 3 did not attempt to do the follow-on work needed to validate that correlation, it correctly and properly indicated that "one cannot conclude [from the work in Example 3] that there are tags in these two samples that are indicators for various parameters associated with each sample". The tags identified and characterized in Example 3 certainly, however, were useful in distinguishing the samples from each other.

The subsequent steps of the invention that were not examined in the work reported in Example 3, i.e., the steps of determining the correlation between the abundance of the nucleic acid tags to the abundance of environment parameters, generating probes specific to gene sequences identified from the tag sequence, and utilizing the probes as a diagnostic or to prospect for the parameter of interest, are disclosed in detail throughout the specification. See, e.g., page 24, line 7 to page 30, line 1.

In particular, the step of determining the correlation between the abundance of the nucleic acid tags to the abundance of environment parameters is described, e.g., at page 27, lines 8-11: "Correlation analysis between a marker (variable 1) and a sample parameter (variable 2) will identify markers whose presence often, or invariably, coincides with a component present in the samples." Indeed, some types of relationships between markers and sample components (or parameters) are shown in Figure 6.

Figure 6 provides six examples of how to measure the correlation of the abundance of a given nucleic acid marker and environmental parameters. These examples illustrate the different

types of correlation that may be displayed between a marker and a parameter. In addition, Figures 9 and 11 depict flow charts that set out the specific steps needed to correlate a nucleic acid tag generated by SARD to environmental parameters. The Marker Diversity Profile (MDP) of Figures 9 and 11 refers to the population of distinct SARD tags generated from an environmental sample and their respective abundances.

The steps of generating nucleic acid probes specific to gene sequences identified from the marker sequence, and utilizing the probes as a diagnostic or to prospect for the parameter of interest are described, e.g., at page 27, lines 18-28:

> In the cases where a significant correlation is found between a marker and sample parameter of interest, the preferred action is to use the tag sequence information to identify the complete gene sequence. The sequence can then be utilized to identify the species and to identify species-specific probes to verify the correlation.
>
> Species-specific probes that are identified from markers with a robust correlation to a sample parameter of interest can then be utilized as a diagnostic, or to prospect for the parameter of interest. Such assays would preferably be PCR-based and would be highly sensitive, rapid and inexpensive.

The identification of a more complete gene sequence from a tag sequence and the further identification of the organisms characterized by these genes are well known in the art. Furthermore, the generation of nucleic acid probes for use as primers for PCR-based assays, as well as the performance of PCR-based assays, are also well known in the art.

In sum, Example 3 describes the finding of differential tags, and the specification teaches the next steps in the process and provides adequate detail, to enable any persons skilled in the art to carry out the claimed invention.

Confirmation of the enablement of this application is provided in the contemporaneously filed Declaration of Matthew Ashby Under 37 CFR 1.132 ("the Declaration"). In the

Declaration, applicant discloses how, using the processes described in this application, applicant determined the correlation between the abundance of nucleic acid tags (generated in accordance with this invention) to specific environment parameters, generated nucleic acid probes specific to gene sequences identified from those tag sequences, and utilized the probes as a diagnostic or to prospect for the parameter of interest.

The Declaration describes, in particular, experiments conducted under the direction and control of applicant, and demonstrates the successful generation and determination of SARD tags that correlate with geochemical parameters, i.e., the abundance of metals (copper and aluminum; see the Declaration, paragraph 5) and hydrocarbons, (i.e., propane; see the Declaration, paragraph 10). Further, the Declaration describes applicant's generation of probes for detecting geochemical parameters, and successfully using the probes to identify the location of subsurface natural gas deposits (see the Declaration, paragraph 11).

As evidenced in the Declaration, applicant, using SARD (Example 1 of the application), generated 1,664 non-identical tags from 21 different soil samples (see Declaration, Exhibit A). Applicant then, following the process described in the specification, correlated the abundances of those tags to the abundances of two environmental parameters – aluminum and copper (see the Declaration, paragraph 5). This result is illustrated in Exhibits A1 and A2. In those Exhibits, the abundances of the metal in each of the 21 samples is shown to correlate to the abundances of tags within a related cluster of tags(see the Declaration, paragraph 5). Indeed, samples that had a high concentration of the metal had a high abundance of specific tags in a cluster of tags. Conversely, samples having low metal concentration had low tag abundance (see the Declaration, Exhibits A1 and A2). Those experiments and results alone show that the claims of

this application are enabled. As shown in the Declaration, however, applicant went further. He used the metal-characterizing tags to identify the organisms to which the tags were specific (see the Declaration, paragraph 6). The organisms identified through the copper-characterizing tags, and their relatives, are known to have associations with copper (see the Declaration, paragraph 6). This work is further confirmation of the operability of the processes described and claimed.

Further, as shown in the Declaration, applicant demonstrated the environmental parameter characterizing tags could be used to recover longer fragments of the corresponding 16S rRNA gene and to design PCR primers from those genes (see the Declaration, paragraph 11). The Declaration then showed that those primer pairs were useful in diagnosing or prospecting for propane (an environmental parameter) in various soil samples (see the Declaration, paragraph 11). Such "prospecting", in accordance with the processes of this invention, permitted a quantitative map of the location of the abundance of the parameter within a gas field area (see the Declaration, paragraph 11 and Exhibit B).

The Declaration, thus, provides further evidence of the enablement of the application in the context of the claimed methods.

Contrary to the above actual and specific evidence, the Examiner contends that the invention cannot be practiced as claimed, because it is not possible to reliably determine the abundance of different organisms in environmental samples. The Examiner refers to Witzingerode et al. (FEMS Microbiol. Rev., 21:312-229, 1997; "Witzingerode") and Colbert et al. (Appl. Env. Microbio., 59: 2056-2063, 1993; "Colbert") as supposed support for this argument. Applicant traverses.

Witzingerode reports that the processes leading to and including the PCR amplification of environmental samples potentially introduce error in calculating the final number organisms detected. Witzingerode further reports that because the number of ribosomal RNA operons per genome varies between different bacterial species, the abundance of different bacterial species cannot be estimated from the abundance of different rRNA gene sequences. Neither "problem", relied on by the Examiner, can be used to argue that the claimed process is not enabled.

First and foremost, the potential sources of error, recited in Witzingerode, can be mitigated by the use of appropriate techniques and conditions known in the art. For example, Witzingerode discusses the potential presence of PCR inhibitors such as humic acid. However, Witzingerode itself points out that such PCR inhibitors may be removed from genomic DNA samples by a number of means including agarose gel purification or Qiagen columns (p. 216, end of paragraph 4) and those that are not removed may be inactivated by the addition of BSA or gp32 (p. 217, paragraph 4).

Other potential sources of error, such as differential amplification of templates and chimera formation can also be effectively mitigated (and are routinely mitigated by the skilled worker) by employing appropriate amplification conditions such as: 1) increasing time of denaturing step; 2) decreasing number of PCR cycles; and 3) increasing the template concentration (*See*, e.g., Acinas et al., (2005) Appl Environ Microbiol 71:8966, Lueders and Friedrich (2003) Appl Environ Microbiol 69:320, and Polz and Cavanaugh (1998) Appl Environ Microbiol 64:3724).

In addition, the variation in copy number of the ribosomal RNA (rRNA) operons in microbes, pointed to by Witzingerode and the Examiner, is not related to the claimed process.

Such variation only has the effect of preventing the accurate conversion of the number of copies of a given 16S rRNA gene sequence (or SARD tag) into an absolute number of microbial cells in a given sample. This supposed limitation has no bearing on the claimed process. The claimed process looks to a relative difference in the number of copies of a specific gene sequence in one sample vs. other samples. For example, if applicant identified 1000 copies of a specific 16S rRNA tag in Sample A, and detects 2000 copies of the same tag in Sample B, he can state there are twice as many tags in Sample B compared to Sample A. In the claimed invention, such relative differences in the abundance of tags between samples is the basis for determining correlations between the tags and environmental parameters. Therefore, the claimed invention is independent of the actual number of ribosomal RNA operons present in a given microbial genome. For this reason also, Witzingerode does not affect the enablement of the claimed invention.

Colbert recites that, following the introduction of salicylate, the number of microbial cells increased, then remained constant. The Examiner interprets this report to mean that making a correlation between the presence of such substances and the number of bacteria would be impossible. First, the Declaration shows that the Examiner's assertion is mistaken. Second, the observation made in Colbert is not relevant to the claimed invention because the experiments in Colbert were only conducted over a short time period, i.e., 5-7 days. By contrast, the differences that take place in environmental microbial communities are a result of natural selection. They would, thus, be expected to occur over much longer time periods. Colbert's observations that cells only increased or remained constant would be an exception, rather than a general phenomenon in nature because microbes are an integral component of the carbon cycle (emphasis on cycle). If there were no turnover of dormant cells, the world's biomass would

perpetually increase, which it does not. Bacteria serve as the foundation for many trophic levels and have numerous predators that can remove non-growing cells such as phage, predatory bacteria, protists, etc. Therefore, Colbert does not affect the enablement of the claimed invention.

For all of the above reasons, applicant requests that the Examiner reconsider and withdraw the enablement rejection.

Claim interpretation

The Examiner asserts that the terms "perfect correlation", "high degree of correlation" and "moderate degree of correlation" have not been defined. Applicant respectfully traverses.

As was explained in the response to the October 18, 2007 Office Action, page 29, lines 15-17 of the specification defines moderate correlation ("r is 0.5 to 0.7"), and high correlation ("r is 0.8 to 0.99"). These are not "possible" or "may be" definitions. They are "is" definitions. The definition of perfect correlation as being r=1 is also not a "may be" definition. Rather, it is a standard definition. However, to expedite prosecution, applicant has defined these terms within the claims where correlation is recited (see claims 47-49).

35 U.S.C. §102 – Anticipation

Claims 45-49 and 57 stand rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Wikstrom (J. Biotechnol., 52: 107-120, 1997; "Wikstrom"). The Examiner contends that Wikstrom recites a culture-independent method for determining the abundance of polycylclic aromatic hydrocarbons (PAHs) by determining the abundance of an active catechol 2,3-dioxygenase gene. Applicant traverses.

The claimed invention teaches identifying nucleic acid markers that correlate to environmental parameters without regard to any prior information as to the identity or function of the microorganism from which they are derived, and using the abundance of the marker to diagnose or prospect for the presence and abundance of the environmental parameter. In contrast, Wikstrom recites choosing a gene on the basis of its function, rather than on its correlation to a parameter (i.e. PAHs are substrates for catechol 2,3-dioxygenase). Moreover, Wikstrom does not recite inferring the presence of PAH's from the abundance of 2,3-dioxygenase genes. Rather, it recites using 2,3 dioxygenase genes as "potentially good targets for monitoring bacterial subpopulations involved in ring-cleavage of aromatics and the final steps in degradation of some PAHs" (p. 108, paragraph 3). For both of these reasons, applicant requests that the Examiner reconsider and withdraw the anticipation rejection.

CONCLUSION

Applicant requests consideration of the amended claims in view of the foregoing remarks and that the Examiner allows pending claims 45-50 and 57.

Should the Examiner feel that a telephone conference with applicant's representative would be helpful, she is invited to telephone the undersigned at any time.

Respectfully submitted,

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